ENALAPRILAT IMPROVES SYSTEMIC AND MESENTERIC BLOOD FLOW DURING NORMOTENSIVE RESUSCITATION FROM HEMORRHAGIC SHOCK IN DOGS

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By

Abby Eileen Fiedler

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An Abstract of a Thesis by

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Resuscitative interventions that improve systemic and mesenteric perfusion without causing instability in systemic arterial pressures may be helpful for improving trauma patient outcomes. Blocking angiotensin II formation with enalaprilat may be such an intervention. In this study involving resuscitation from hemorrhagic shock in dogs, the systemic and mesenteric cardiovascular effects of administering enalaprilat during resuscitation were investigated. Thermodilution-based and ultrasound-based methods of monitoring the systemic blood flow were also examined. Animals were hemorrhaged to a mean arterial pressure (MAP) of 40-45mmHg for 30min then 30-35mmHg for 30min followed by resuscitation with intermittent lactated Ringer's solution (200ml/kg/hr during first 40min, 60ml/kg/hr during following 130min) to reach and maintain a mean arterial pressure of 75–80mmHg for 170min. A constant rate infusion of saline vehicle with or without enalaprilat (0.02mg/kg/hr) was initiated after 40min of resuscitation. Systemic and mesenteric blood flows declined with hemorrhage, increased in all dogs with resuscitation, and then declined during the initial 40min of resuscitation. Enalaprilat administration resulted in increases in systemic and mesenteric blood flows not seen in the controls. The greater flows with enalaprilat appeared to be due to prevention of the

increases in afterload noted in the controls. Comparison of monitoring techniques, showed stronger correlation between the ultrasonically determined descending thoracic aortic blood flow and celiac artery flow than between thermodilution determined cardiac output and celiac artery flow. It can be concluded that administration of a constant rate infusion of enalaprilat during resuscitation can be used to improve systemic and mesenteric blood flow and that passive methods of monitoring systemic blood flow, such as transesophageal ultrasound, may more accurately represent flow parameters than do bolus requiring methods, such as intermittent thermodilution.

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INTRODUCTION

Hemorrhage leads to reductions in systemic and, even more so, in mesenteric perfusion (Toung et al., 2000). Restoration of systemic cardiovascular parameters after trauma, however, is not always accompanied by restoration of gastrointestinal energy status (Ivatury et al., 1996; Van Way et al., 1996). Patients in whom gastrointestinal energy status is normalized more quickly have a decreased incidence of multiple organ dysfunction and death (Ivatury et al., 1996; Chang et al., 1994; Kirton et al., 1998). Accordingly, early institution of resuscitative interventions that improve not only global systemic cardiovascular parameters, but also mesenteric blood flow may be beneficial.

Angiotensin II has been shown to be responsible for the disproportionate mesenteric vasoconstriction that occurs in response to hemorrhage (Toung et al., 2000). Administration of an angiotensin converting enzyme inhibitor during resuscitation would be a reasonable approach for increasing mesenteric blood flow. Benefits from interference with angiotensin II-mediated processes before, during, and after shock have been reported (Toung et al., 2000; Åneman et al., 1997; Wall et al., 1999; Kincaid et al., 1998; Åneman et al., 2000; Wall et al., 2002). A smaller decrease in mesenteric perfusion in pig hemorrhagic shock models resulted when the angiotensin converting enzyme inhibitor enalaprilat (Merck Company Inc.) was administered before hemorrhage (Toung et al., 2000; Åneman et al., 1997). A greater increase in metabolic activity, indicated by increasing body temperature (Henderson et al., 1999), and a greater survival to 48 hours (Wall et al., 1999) occurred when enalaprilat was administered during resuscitation in a rat hemorrhagic shock and resuscitation model. In human trauma patients, an increase in cardiac output and a decrease in gastric P_iCO_2, indicating

improved gastric mucosal energy status, resulted when enalaprilat was administered during resuscitation (Kincaid et al., 1998). Despite an intentionally hypotensive resuscitation protocol, administration of enalaprilat to dogs during resuscitation from hemorrhage was also associated with an increase in cardiac index as well as increases in superior mesenteric artery and portal vein blood flow, all without adversely affecting systemic arterial pressure maintenance (Wall et al., 2002).

In addition to angiotensin converting enzyme inhibition, angiotensin II sub-type 1 receptor blockade has also shown benefits. A smaller decrease in mesenteric perfusion and increased survival occurred in a pig model of hemorrhagic shock and resuscitation when candesartan, an angiotensin II sub-type 1 receptor blocker was administered before hemorrhage (Åneman et al., 2000). Angiotensin II sub-type 1 receptor blocker administration during resuscitation of trauma patients, however, has not been reported. Not all drugs that prevent either formation of angiotensin II or interaction of angiotensin II with receptors are likely to be equally beneficial (Guba et al., 2000; Schindler et al., 1995).

This study investigated the systemic and mesenteric cardiovascular effects of administering a constant rate infusion of enalaprilat during normotensive resuscitation from hemorrhage with lactated Ringer's solution. This is a continuation from previous work which demonstrated that systemic arterial pressures could be maintained while administering a constant rate infusion of enalaprilat during moderately hypotensive resuscitation, and that administering a constant rate infusion of enalaprilat during severely hypotensive resuscitation improved systemic cardiovascular status and, importantly, mesenteric blood flow despite the severe hypotension (Wall et al., 2002). In addition, this

study compared two methods of assessing systemic blood flow: intermittent thermodilution using a pulmonary artery catheter and ultrasound using a transesophageal combined M-mode and pulsed Doppler ultrasound probe.

MATERIALS AND METHODS

This research was conducted in accordance with the: National Institutes of Health guidelines for experiments involving animals, provisions of the USDA Animal Welfare Act, Animal and Plant Health Inspection Services (APHIS) Guide for the Care and Use of Laboratory Animals, and US Interagency Research Animal Committee Principles for the Utilization and Care of Research Animals. The protocols were approved by the Animal Care and Use Committee, Veterans Administration Central Iowa Health Care System, Des Moines, Iowa. All experiments were undertaken in the Veterans Administration Central Iowa Health Care System Surgical Teaching Laboratory, an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility.

Animal Model

Mongrel dogs (21.8 ± 0.5 kg, City of Des Moines Animal Shelter) were used for all experiments. The night prior to surgery, food was withheld. Water was available *ad libitum*. Dogs were randomized to enalaprilat or control before anesthesia and the group to which they were randomized was kept concealed until all ten had been completed. Blinding was maintained by having technicians who were not otherwise involved in the study randomize the dogs and prepare the appropriate enalaprilat in saline (0.07% physiological saline, Wall et al., 2002, hereafter referred to as saline) solution or saline

alone solution for administration during resuscitation. All of the dogs that survived hemorrhage and resuscitation were euthanized with 0.22 ml/kg of Euthasol® (Delmarva laboratories, Midlothian, Virginia, USA) administered intravenously at the end of resuscitation. The minimum number of subjects per treatment was determined by power analysis (GraphPad Prism version 3.02 for Windows, GraphPad Software, San Diego, CA and SAS®, SAS Institute, Inc., Cary, NC). Previous work by Wall et al., 2000 demonstrated a 20% loss.

Anesthesia

Animals were premedicated with xylazine (1 mg/kg, intramuscularly, Phoenix Pharmaceutical Inc.) and atropine (0.8 mL intramuscularly, American Pharmaceutical Partners), induced with thiopental (Abbott Laboratories), and kept anesthetized with a constant rate infusion of thiopental. Local anesthesia in the form of 1.0% lidocaine (Baxter Health Care Inc.) was also used at incision sites.

Instrumentation

The locations and purposes of the items placed in the dogs are found in Table 1. Except for the left cephalic vein, cut-downs were used to place the vascular catheters, and the incisions were sutured closed. Normal saline was run through the left brachial arterial catheter at 1 ml/kg/h. Lactated Ringer's solution was run through the other three arterial catheters at 0.2 ml/kg/hr. The intra-abdominal catheters and sensors were placed via laparotomy. The laparotomy was closed with towel clamps after instrumentation so that

1 cm^3 hepatic and gastric mucosal samples could be obtained at several time points in each dog and preserved for later analysis.

Hemorrhage

After obtaining baseline measurements, dogs were bled to and maintained at a mean arterial pressure of 40 – 45 mm Hg for 40 min (Figure 1). The end of the first hemorrhage

samples and measurements were obtained during the last 10 min of this first interval. During the second hemorrhage dogs were bled to and maintained at a mean arterial pressure of 30 – 35 mm Hg for 40 min. The end of the second hemorrhage samples and measurements were obtained during the last 10 min of the second hemorrhage interval. Then resuscitation commenced. Resuscitation was begun earlier if the mean arterial pressure dropped below 30 mm Hg for 10 min, or below 25 mm Hg for 1 min. In the event that resuscitation was started early, the appropriate samples and measurements were obtained as rapidly as possible and were considered to represent both the end of the second hemorrhage and the start of resuscitation.

Table 1. Instrumentation

Item	Location	Purpose
21 gauge butterfly extension	left cephalic vein	induction and constant rate infusion of thiopental for anesthesia
endotracheal tube	trachea	airway control
ventilator	near head of dog	SIMV at tidal volume=8-12mL/kg and rate=25-45 breaths/min for P_aCO_2=35-45mmHg
mainstream end-tidal CO_2 monitor	attached to endotracheal tube	end-tidal CO_2 and respiratory rate
pulse oximeter	tongue	SpO_2
esophageal stethoscope	esophagus	cardiac auscultation
transesophageal M-mode and pulsed Doppler ultrasound probe (Hemosonic®, Arrow International, Reading, PA)	esophagus	left ventricular ejection time interval and descending thoracic aortic: 1. blood flow 2. stroke volume 3. diameter 4. blood maximal acceleration 5. blood peak velocity
ECG leads	skin	ECG and heart rate
7.5 Fr thermodilution catheter[a]	right external jugular vein to pulmonary artery	CVP, PAP, PAOP, CO, mixed venous samples for S_vO_2
8 Fr 2 lumen catheter	right brachial artery	bleeding, systemic arterial pressures back-up site
12 Fr 2 lumen catheters	1. right femoral artery 2. left femoral artery	systemic arterial pressures, bleeding back-up sites
7 Fr 3 lumen catheter	right femoral vein	resuscitation fluids
Foley catheter	bladder	collecting urine
4 Fr 1 lumen catheters	1. left brachial artery 2. upper thoracic esophagus 3. through gastric wall 4. through duodenal wall	fiber-optic sensor placements
fiber-optic sensors[b]	1. left brachial artery 2. esophageal lumen 3. gastric lumen 4. duodenal lumen	1. continuous pH_a, P_aCO_2, P_aO_2, BE, S_aO_2 2. eP_iCO_2 3. gP_iCO_2 4. dP_iCO_2
transit time ultrasound flow probes[c]	1. around the celiac artery 2. around the portal vein	1. celiac artery blood flow 2. portal vein blood flow

[a]Arterial pressures and cardiac output were monitored using a SpaceLabs Medical PC2 Bedside Monitor (SpaceLabs Medical, Redmond, Washington, USA).

[b]Fiber-optic sensors were Neotrends® (Diametrics Medical, St. Paul, Minnesota, USA).

[c]Celiac arterial flow and portal vein flow were monitored using a Transonics® T206 monitor (Transonics Systems Inc., Ithaca, New York, USA).

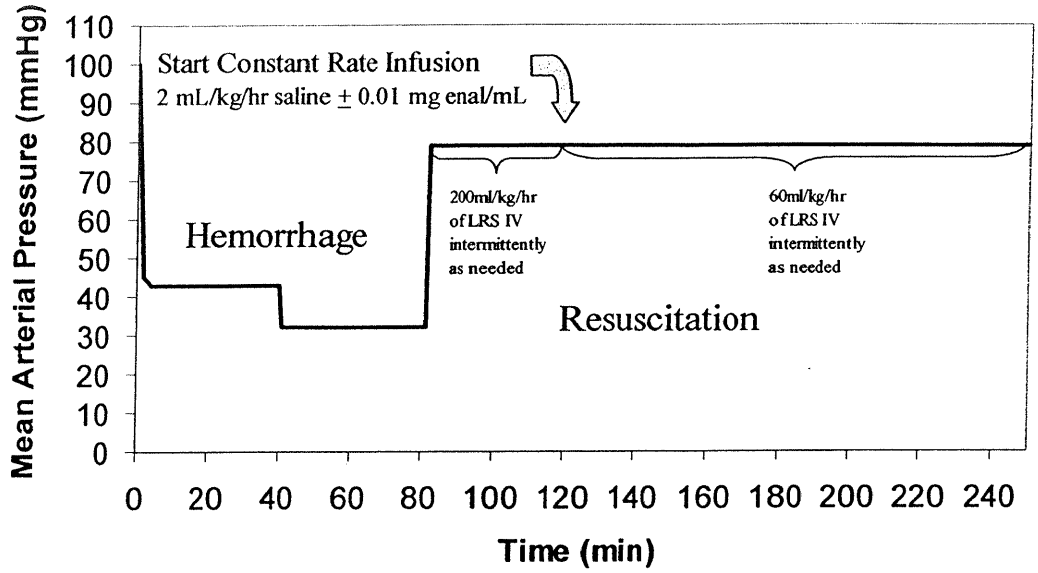

Figure 1. Hemorrhage and resuscitation protocol. The line represents the mean arterial pressure (MAP) (shown on the y-axis) over time from the baseline (shown on the x-axis).

Resuscitation

After hemorrhage, each dog was resuscitated with intermittent intravenous lactated Ringer's solution at 200 mL/kg/hr titrated to reach and maintain mean arterial pressure at 75 - 80 mm Hg (Figure 1). After the first 40 min interval, the rate of lactated Ringer's solution for intermittent administration was reduced to 60 ml/kg/hr and a constant rate infusion of either saline vehicle with enalaprilat (0.02 mg enalaprilat/kg/hr, n = 5) or without enalaprilat (n = 5) was administered intravenously for 130 min. The saline vehicle infusion rate for both treatment and control animals was 2 mL/kg/hr. Resuscitation samples and measurements were taken at the start of resuscitation, after 30 min of resuscitation, at the start of the constant rate infusion of the saline vehicle with or without enalaprilat, and every 30 min following the initiation of the constant rate infusion.

Measurements And Samples

Descending thoracic aortic blood flow variables, systemic arterial pressures, pulmonary arterial pressures, central venous pressure, heart rate, SpO_2, P_aO_2, P_aCO_2, pH_a, and BE_a were continuously monitored and were recorded at 1 min intervals. Arterial blood samples were taken for blood gas analysis and hemoglobin determination at baseline, the start of resuscitation, and the end of resuscitation. Mixed venous blood samples were taken at baseline and at the end of each hemorrhage and resuscitation interval. Using a pulmonary artery thermodilution catheter, cardiac output and pulmonary artery occlusion pressure were measured at baseline and at the end of each hemorrhage and resuscitation interval. Ten mL cold saline was used as the injectate bolus for determining cardiac output. Cardiac index (CI) and stroke volume index (SVI) were calculated from the cardiac output and heart rate values. The body surface area calculation used for indexing was as follows: body surface area (m^2) = [weight $(kg)^{0.67} \times 10.1]/10^2$ (Chang et al., 2000). Stroke work index (SWI; Reilly et al., 2001), left ventricular power output index (LVPI; Yamazaki et al., 1991; ATLS 1997), and arterial elastance index (E_aI; Reilly et al., 2001) were calculated using the following equations: SWI = SVI × femoral mean arterial pressure; LVPI = CI × (femoral mean arterial pressure – central venous pressure); E_aI = femoral mean arterial pressure/SVI. Aortic blood flow index and aortic stroke volume index were calculated from the aortic blood flow and the heart rate values.

Statistical Analysis

Quantitative data are reported as means ± the standard errors of the mean (SEM). Data was analyzed using two-way repeated measures ANOVA using commercially available statistical software programs (GraphPad Prism version 3.02 for Windows, GraphPad Software, San Diego, CA and SAS®, SAS Institute, Inc., Cary, NC). A p value of less than 0.05 was considered statistically significant.

RESULTS

Hemorrhage

Hemorrhage decreased blood pressure, cardiac index, and descending thoracic aortic, celiac arterial, and portal venous blood flow (Figures 2a,2b,2c, 3a,3b, and Table 2). The severity of the metabolic insult caused by the hemorrhage is indicated by the decreases in arterial base excess and S_vO_2 (Table 2), increases in gastrointestinal P_iCO_2 (Table 2), and the pre-resuscitation death of two dogs, one from each group.

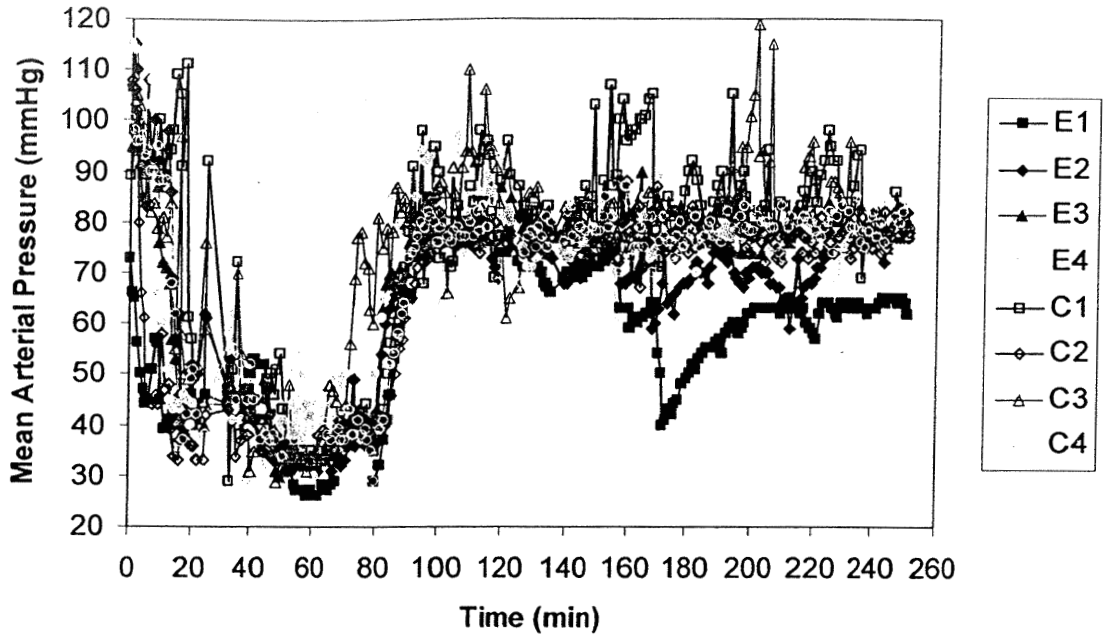

Figure 2a. Mean arterial pressure during hemorrhage and resuscitation with and without enalaprilat in each dog. Hemorrhage started at time = 1 minute, resuscitation at time = 81 minutes, constant infusion rate of enalaprilat or control saline vehicle at time = 121 minutes. Abbreviations: E = enalaprilat group, C = control group, numbers indicate individual animals.

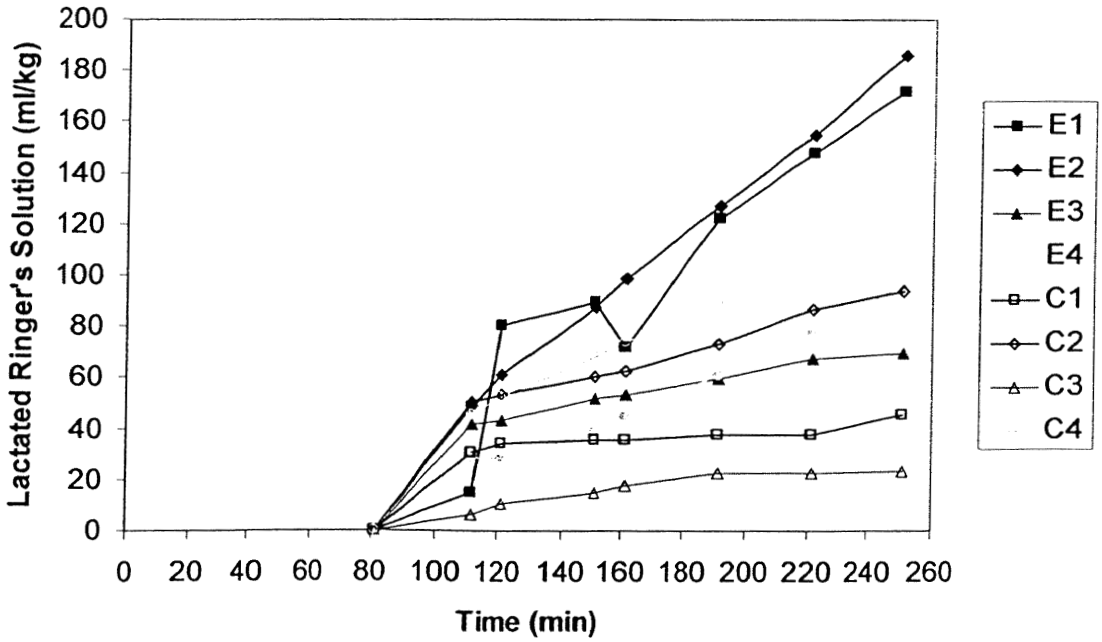

Figure 2b. Volumes of lactated Ringer's solution administered during resuscitation with and without enalaprilat to each dog. Hemorrhage started at time = 1 minute, resuscitation at time = 81 minutes, constant infusion rate of enalaprilat or control saline vehicle at time = 121 minutes. Abbreviations: E = enalaprilat group, C = control group, numbers indicate individual animals.

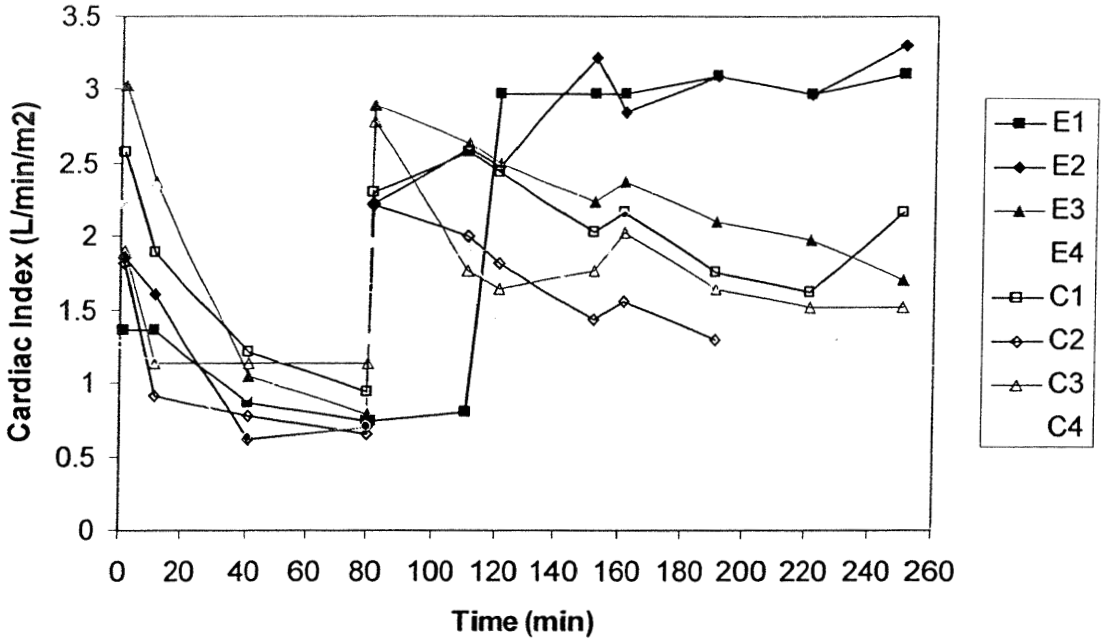

Figure 2c. Cardiac indices during hemorrhage and resuscitation in each dog. Data from animals that received enalaprilat are shown with solid markers, and data from control animals are shown with open markers. Hemorrhage started at time = 1 minute, resuscitation at time = 81 minutes, constant infusion rate of enalaprilat or control saline vehicle at time = 121 minutes. Abbreviations: E = enalaprilat group, C = control group, numbers indicate individual animals.

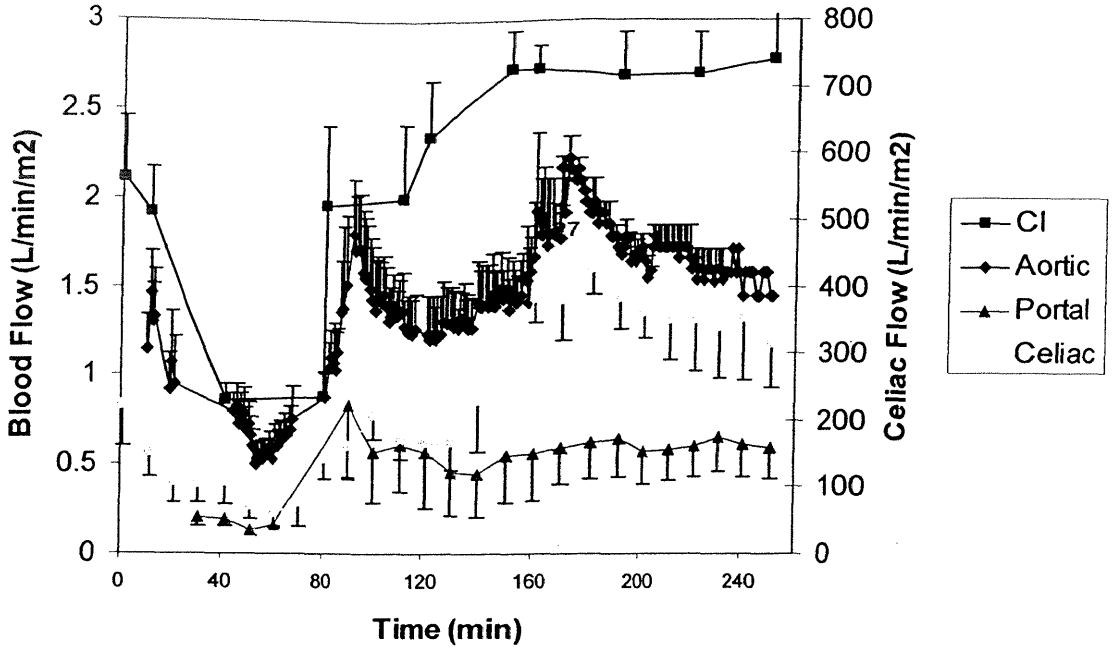

Figure 3a. Average cardiac indices and decending thoracic aortic, portal venous, and celiac artery blood flow indices during hemorrhage and resuscitation in the enalaprilat group. Hemorrhage started at time = 1 minute, resuscitation at time = 81 minutes, constant rate infusion of enalaprilat or control saline vehicle at time = 121 minutes. Enalaprilat descending thoracic aortic blood flow index values from 150 min to 203 min on the x-axis represent values from only 3 dogs. Enalaprilat descending thoracic aortic blood flow index values from 203 min to 233 min on the x-axis represent values from only 2 dogs. Enalaprilat descending thoracic aortic blood flow index values from 233 min onward represent values from only 1 dog. Control cardiac index values beyond 191 min on the x-axis represent values from only 3 dogs. Control descending thoracic aortic blood flow index values from 242 min onward represent values from only 3 dogs. Abbreviation: CI = cardiac index.

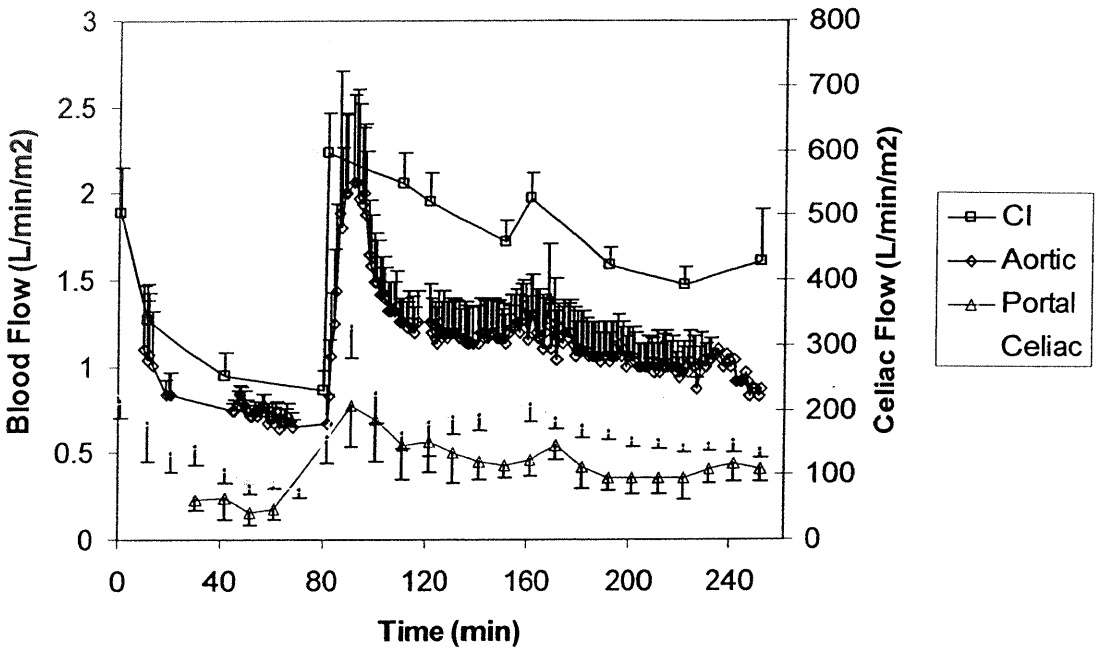

Figure 3b. Average cardiac indices and decending thoracic aortic, portal venous, and celiac artery blood flow indices during hemorrhage and resuscitation in the control group. Hemorrhage started at time = 1 minute, resuscitation at time = 81 minutes, constant rate infusion of enalaprilat or control saline vehicle at time = 121 minutes. Enalaprilat descending thoracic aortic blood flow index values from 150 min to 203 min on the x-axis represent values from only 3 dogs. Enalaprilat descending thoracic aortic blood flow index values from 203 min to 233 min on the x-axis represent values from only 2 dogs. Enalaprilat descending thoracic aortic blood flow index values from 233 min onward represent values from only 1 dog. Control cardiac index values beyond 191 min on the x-axis represent values from only 3 dogs. Control descending thoracic aortic blood flow index values from 242 min onward represent values from only 3 dogs. Abbreviation: CI = cardiac index.

Resuscitation

Since the administration of lactated Ringer's solution was titrated so as to maintain the mean arterial pressure in the resuscitation target range of 75 - 80 mm Hg, the dogs received varying amounts of lactated Ringer's solution throughout the course of resuscitation (Figure 2b). During the intitial 40 minutes of resuscitation, there was not a significant difference in requirements for lactated Ringer's solution between groups (53 ± 11 ml/kg in enalaprilat group versus 38 ± 10 ml/kg in control group, p=0.4). Both groups also had increases in systemic and mesenteric blood flows with the initiation of resuscitation. Over time, however, systemic and mesenteric blood flows declined in the control dogs and ended higher in the enalaprilat dogs (Figures 3a and b). The data in Table 2 show that the changes in systemic blood flow were the result of changes in stroke volume, not heart rate.

Following initiation of the constant rate infusion at 40 minutes into resuscitation (120 minutes into the protocol), two of the control dogs took very little to no additional lactated Ringer's solution. These two control dogs generally had resuscitation mean arterial pressures greater the 80 mm Hg (Figures 2a,b,c; C1, C3). The other two control dogs and two of the enalaprilat dogs had relatively indistinguishable mean arterial pressures (Figures 2a,b,c; C2, C4, E3, E4). Following initiation of the constant rate infusion, these two control dogs (C2, C4) required 40.3 and 51.2 ml/kg lactated Ringer's solution, and the two enalaprilat dogs (E3, E4) with similar mean arterial pressures required 26.4 and 68.2 ml/kg lactated Ringer's solution. Over this time frame, these two enalaprilat dogs (E3, E4) generally had greater cardiac indices than any of the control dogs.

The other two enalaprilat dogs' (E1, E2) mean arterial pressures were not well maintained in the target range of 75 - 80 mm Hg following initiation of the constant rate infusion despite continuous administration of lactated Ringer's solution. Though dog E1 had mean arterial pressures below the target range throughout the constant rate infusion period, a fluid pump error likely contributed. Dog E1 received only 2,087 mL of lactated Ringer's solution but should have received up to 390 additional millilters of lactated Ringer's solution. These two enalaprilat dogs (E1, E2), however, had the highest cardiac indices and stroke volume indices throughout the constant rate infusion period (Figure 2c and Table 2).

Table 2. Systemic measurements during hemorrhage and normotensive resuscitation with LRS

	Baseline	End 1st H	End 2nd H	40min R	80min R (40min enal)	110min R (70min enal)	140min R (100min enal)	170min R (130min enal)
MAP mmHg								
control	101±4	43±4	44±6	79±7	81±6	82±1	80±1	80±2
[a]enalaprilat	98±11	44±2	39±5	81±3	72±5	70±5	72±5	74±4
HR beats/min								
control	135±4	124±18	151±17	132±9	123±8	138±9	138±10	132±10
[a]enalaprilat	127±8	129±7	141±14	123±16	119±13	130±13	127±12	125±11
PAOP mm Hg								
control	3±2	-1±1	-1±1	0±1	1±1	1±1[b]	2±1[c]	1±1[b]
enalaprilat	7±2	2±2	2±2	2±3	4±3[b]	1±3[b]	3±3	-2±3[c]
SVI mL/m^2								
control	14±2	9±2	6±1	15±2	15±0	11±1	10±1	12±3[b]
[a]enalaprilat	17±3	7±1	6±1	20±4	22±3	22±4	22±3	23±4
ASVI mL/m^2								
control	8±1	6±1	4±1	10±1	9±1	7±1	7±1	7±1[b]
[a]enalaprilat	11±2	6±1	6±1	11±2	16±3	14±1[b]	12±0[c]	11[d]
BE_v mEq/L			-	-				
control	-4.9±0.4	-8.2±1.2	12.0±1.5	11.2±1.6	-9.1±1.1	-8.5±0.8	-7.5±1.5[b]	-7.9±1.2
enalaprilat	-5.1±0.3	-8.6±1.1	- 12.1±1.0	-8.5±1.2	-8.0±1.3[b]	-6.5±1.0	-6.2±1.0	-6.4±0.6
S_vO_2 %								
control	66±3	34±6	23±5	44±2	43±5	44±8	35±4[b]	37±7[b]
[a]enalaprilat	69±4	38±5	20±3	50±7	53±6	47±4	46±3	47±3
DO_2I mL/min/m^2								
control	365±54	--	124±21	--	--	--	--	147±30
enalaprilat	405±56	--	120± 9	--	--	--	--	198±23
VO_2I mL/min/m^2								
control	110±7	--	91±10	--	--	--	--	90±11
enalaprilat	113±3	--	90± 5	--	--	--	--	105±14
Hb g/dL								
control	15.0±0.6	12.4±0.7	11.2±0.9	8.5±0.5	7.9±0.4	6.7±0.7	8.0±0.5	7.3±0.3
enalaprilat	14.8±0.5	13.0±0.6	11.1±1.0	7.9±0.1	6.8±0.5	6.4±0.5	5.9±0.5	5.6±0.6
Urine mL/kg[e]								
control	0	0.5±0.3	0.6±0.3	1.0±0.2	2.2±0.8	3.5±1.4	3.8±1.3	4.6±1.8
enalaprilat	0	0.4±0.2	0.5±0.2	1.1±0.3	2.8±0.9	3.8±1.0	4.2±0.9	5.1±1.4
eP_iCO_2 mmHg								
control	56±4	70±6	75±6	58±4	51±3	49±4	49±5	48±5
enalaprilat	69±10[b]	76±12[b]	85±9[b]	64±4[c]	53±1[c]	52±0[c]	50±0[c]	49±2[c]
gP_iCO_2 mmHg								
control	76±5	88±9	108±24	68±6	64±8	59±7	66±9	65±7
enalaprilat	58±10	77±15	117±28	64±10[b]	53±5[b]	47±4[b]	52±9[b]	49±6[b]
dP_iCO_2 mmHg								
control	56±6	75±12	97±34	49±3b	48±4[b]	43±5[b]	44±4[b]	41±4[b]
enalaprilat	100±8[b]	81±8[b]	104±21[b]	65±15[b]	54±4[b]	58±6[b]	63±8[b]	60±6[b]

[a]The p-value ≤ 0.05 verus control following initiation of the constant rate of infusion.

[b]This value is from three dogs.

[c]This value is from two dogs.

[d]This value is from one dog.

[e]The urine volumes are cumulative.

In terms of group averages following the initiation of the constant rate infusion, the enalaprilat group had a lower average mean arterial pressure ($p<0.0001$), required more lactated Ringer's solution (0.002), and had a higher average cardiac index ($p=0.0001$), stroke volume index ($p=0.0014$), celiac arterial flow ($p<0.0001$), and portal venous flow ($p=0.0002$). In the enalaprilat group, the increase in celiac arterial flow following initiation of the constant rate infusion of enalaprilat was quite marked, and its pattern matched that of the ultrasonically determined descending thoracic aortic blood flow better in every dog than it matched that of the thermodilution determined cardiac output (Figure 3a and Table 3).

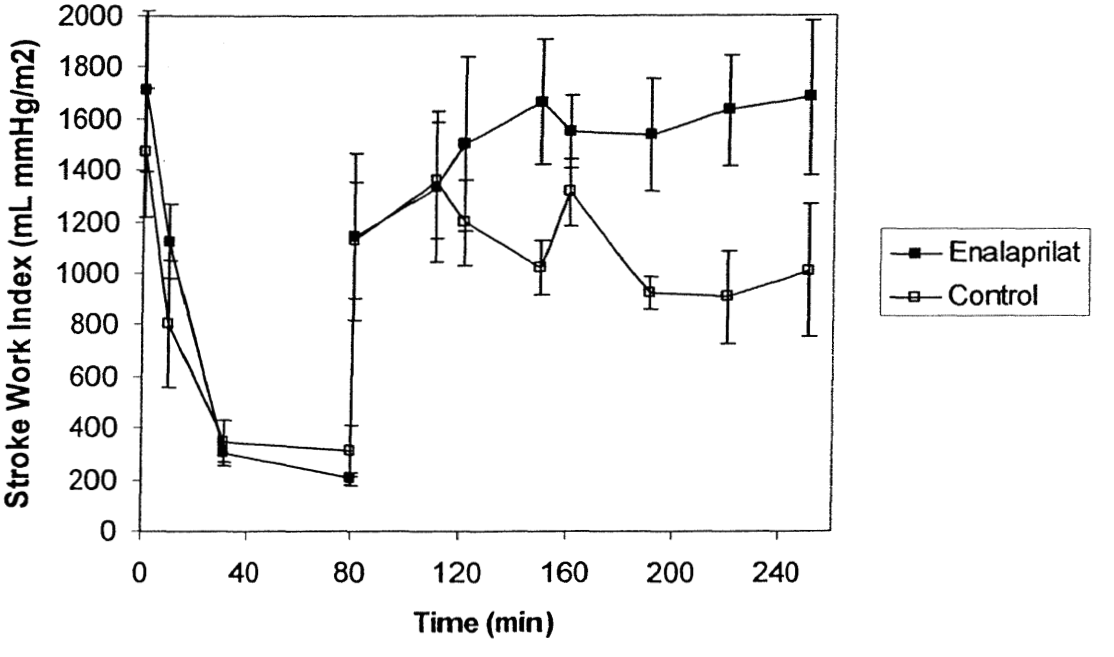

Figure 4a. Average stroke work indices during hemorrhage and resuscitation. The average stroke work indices following the initiation of the constant rate infusion were higher in the enalaprilat group than in the control group ($p = 0.003$). Control values beyond 191 min on the x-axis represent values from only 3 dogs. Hemorrhage started at time = 1 minutes, resuscitation at time = 81 minutes, constant rate infusion of enalaprilat or control saline vehicle at time = 121 minutes.

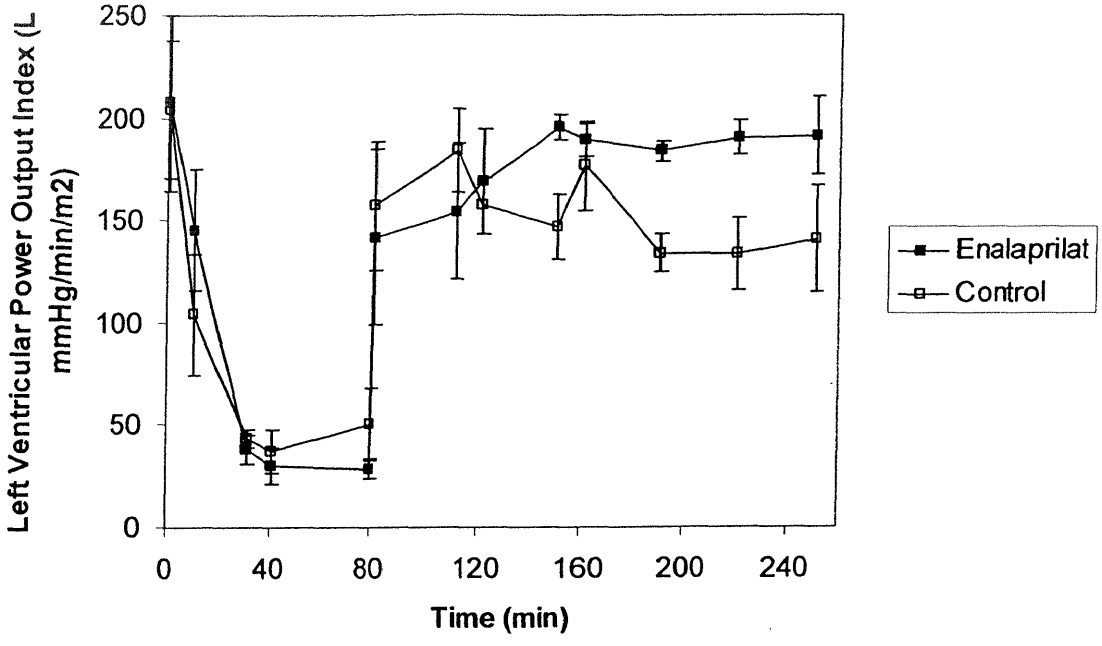

Figure 4b. Average left ventricular power output indices during hemorrhage and resuscitation. The average left ventricular power output indices following initiation of the constant rate infusion were higher in the enalaprilat group than the control group ($p = 0.0094$). Control values beyond 191 min on the x-axis represent values from only 3 dogs. Hemorrhage started at time = 1 minutes, resuscitation at time = 81 minutes, constant rate infusion of enalaprilat or control saline vehicle at time = 121 minutes.

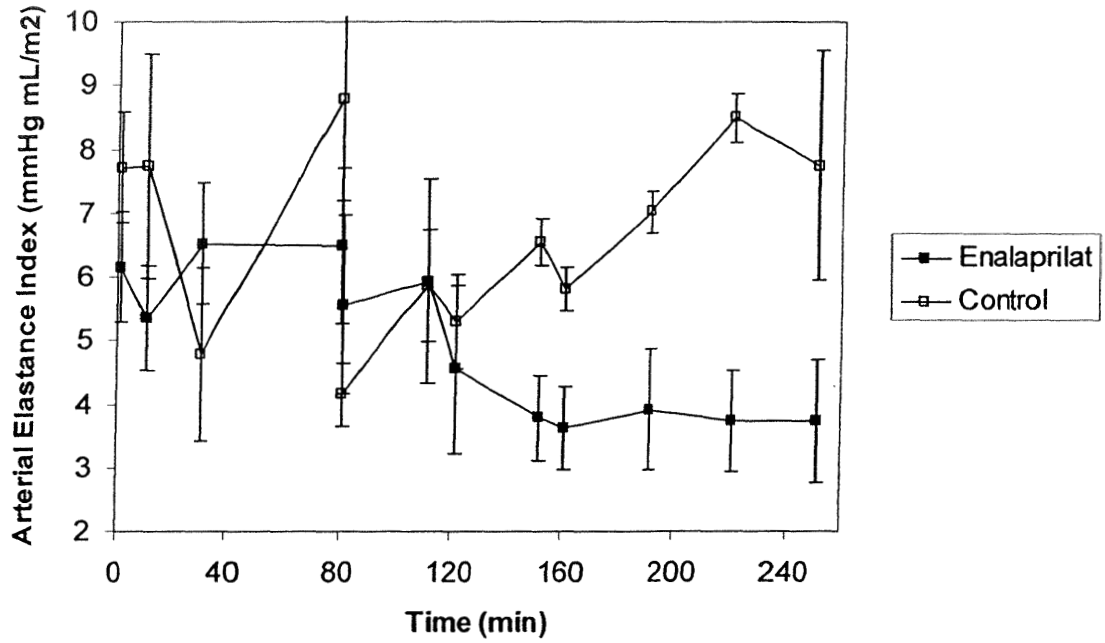

Figure 4c. Average arterial elastance indices during hemorrhage and resuscitation. The average arterial elastance indices following initiation of the constant rate infusion were lower in the enalaprilat group than the control group ($p = 0.0001$). Control values beyond 191 min on the x-axis represent values from only 3 dogs. Hemorrhage started at time = 1 minutes, resuscitation at time = 81 minutes, constant rate infusion of enalaprilat or control saline vehicle at time = 121 minutes.

Table 3. Comparison of Values Obtained for Cardiac Output, Descending Thoracic Aortic Blood Flow, and Celiac Artery Blood Flow Measured by Different Techniques

	Pearson Correlation Coefficients, Number of Time Points Compared		
Dog	CO versus ABF	CO versus Celiac	ABF versus Celiac
Enalaprilat 1	0.78, 10 time points	0.85, 10 time points	0.91, 10 time points
Enalaprilat 2	0.91, 11 time points	0.84, 11 time points	0.91, 11 time points
Enalaprilat 3	0.91, 12 time points	0.74, 7 time points	0.94, 7 time points
Enalaprilat 4	0.85, 6 time points	0.81, 6 time points	0.89, 6 time points
Control 1	0.65, 12 time points	0.32, 12 time points	0.83, 12 time points
Control 2	0.91, 10 time points	0.84, 10 time points	0.94, 10 time points
Control 3	0.19, 12 time points	0.64, 12 time points	0.84, 12 time points
Control 4	0.76, 11 time points	0.80, 10 time points	0.98, 10 time points

Time points for comparison were those at which cardiac output was measured by thermodilution. Abbreviations: CO = cardiac output measured using thermodilution, ABF = descending thoracic aortic blood flow measured using combined M-mode and pulsed Doppler ultrasound, Celiac = celiac arterial blood flow measured using transit time ultrasound.

Systemic Flow-Pressure Related Parameters During Resuscitation

Stroke work index and left ventricular power output index were higher during resuscitation in the enalaprilat dogs than in the control dogs ($p=0.003$ and $p=0.0094$, respectively; Figures 4a,b). Arterial elastance index, a measure of afterload, was lower during resuscitation in the enalaprilat dogs than in the control dogs ($p=0.0001$ enalaprilat versus control; Figure 4c). In the control dogs arterial elastance generally increased during resuscitation.

Esophageal And Gastrointestinal P_iCO_2 During Resuscitation

Declines in P_iCO_2 at each monitored site occurred with the initiation of resuscitation. The P_iCO_2 values in both groups generally reached or were below baseline by the start of the respective constant rate infusions (Table 2). Due to a variety of fiber-optic instrumentation constraints, not all P_iCO_2 values were available in all dogs.

Pulmonary Artery Catheter Knot

Thermodilution dependent values were not obtained in one of the control dogs beyond 110 min of resuscitation (70 min after the initiation of the control constant rate infusion). When an appropriate pulmonary artery trace was lost and repositioning attempted, resistance was encountered and a few premature ventricular contractions occurred. The catheter was then left in place and not used through the remainder of resuscitation in this dog. An intracardiac knot in the pulmonary artery catheter was found on necropsy.

Transesophageal Ultrasound of Descending Thoracic Aortic Flow Parameters

It was discovered that the transesophageal ultrasound probe used to obtain descending thoracic aortic blood flow values requires occasional repositioning if the stomach is manipulated. The loss of well defined aortic walls on the M-mode trace can occur without loss of the audible Doppler derived representation of blood flow, and no data will be collected in the absence of well defined aortic walls on the M-mode. With the version of the Hemosonic used in this study, real time data collection and storage was apparently suspended when the data trends screen was visible. These discoveries came at the expense of some descending thoracic aortic blood flow data (Figures 3b,3c).

DISCUSSION

This study had four major findings. (1) Enalaprilat improved systemic blood flow. (2) Enalaprilat improved mesenteric blood flow. (3) The transesophageal ultrasonic method of monitoring systemic blood flow correlated well with the intermittent thermodilution method. (4) The results with the transesophageal ultrasonic method of monitoring systemic blood flow suggested that it may have advantages over intermittent thermodilution based monitoring independent of its continuous and less invasive nature.

Greater Systemic And Mesenteric Blood Flow During Resuscitation With Enalaprilat

Consistant with previous findings in a hypotensive resuscitation protocol (Wall et al., 2002), the group that received the enalaprilat infusion had greater systemic and mesenteric flows at the end of resuscitation. In this hypotensive resuscitation protocol, the base excess at the start of the constant rate infusion was strongly correlated with the amount of lactated Ringer's solution required irrespective of treatment group, and no difficulty was encountered in maintaining target mean arterial pressures with intermittent administration of lactated Ringer's solution as needed at 60 mL/kg/hr (Wall et al., 2002). In the current resuscitation protocol, with target mean arterial pressures of 75 - 80 mm Hg and intermittent administration of lactated Ringer's solution as needed at 60 mL/kg/hr, the base excess at the start of the constant rate infusion was not strongly correlated with the amount of lactated Ringer's solution required, and two of the dogs that received enalaprilat were not maintained in the target mean arterial pressure range following

initiation of the enalaprilat constant rate infusion. During this period, however, these two dogs had the highest cardiac indices and stroke volume indices of all of the dogs and had higher celiac arterial flows than did the controls. The cardiac indices and stroke volume indices of the other two enalaprilat dogs following initiation of the enalaprilat infusion were also generally higher than any of the controls. They also had increasing celiac arterial flows with the enalaprilat infusion while celiac arterial flows in the controls generally declined during the constant rate infusion period.

Blood Flow Relationship With Blood Pressure

Looking at the systemic and the mesenteric blood flow in concert with the blood pressure reinforces the point that blood pressure is a very poor indicator of blood flow. In fact, even with an average infusion period mean arterial pressure of 41 ± 0 mm Hg, the average final cardiac index in the previous study with hypotensively resuscitated dogs that received enalaprilat was 2.3 ± 0.2 L/min/m^2 (Wall et al., 2002), 0.7 L/min/m^2 greater than the average final cardiac index in the control dogs in this current protocol (average mean arterial pressures during the constant rate infusion period of 81 ± 0 mm Hg). The greater blood flow with enalaprilat was achieved with a volume of lactated Ringer's solution that would otherwise likely have merely increased the mean arterial blood pressure without such a bonus effect on flow. Administration of 101 ± 12 mL/kg lactated Ringer's solution combined with enalaprilat resulted in mean arterial pressures of 40 - 45 mm Hg with improved flows while the administration of 67 ± 19 mL/kg lactated Ringer's solution without enalaprilat resulted in mean arterial pressures of 75 - 80 mm Hg with unimproved flows (Wall et al., 2002).

Cardiovascular Flow-Pressure Parameters During Resuscitation With Enalaprilat

It has been suggested that, for assessing and guiding resuscitative interventions, using variables that combine blood flow information with blood pressure information may have advantages over using variables containing solely blood flow information (Chang et al., 1998; Chang et al., 2000). The flow-pressure parameters in this study (stroke work index, left ventricular power output index, and arterial elastance index) were calculated using the thermodilution obtained systemic blood flow data since thermodilution is currently more commonly employed for monitoring systemic blood flow parameters than is ultrasound. Both stroke work index and left ventricular power output index ended higher in the animals that received enalaprilat while arterial elastance, a measure of afterload, ended lower with enalaprilat. This would suggest that part of the mechanism for the difference in cardiovascular performance is an enalaprilat-induced decrease in afterload (while preload was maintained with fluids). Another contributing factor is the increasing arterial elastance observed in the control group during resuscitation. This would suggest that increasing afterload in the control animals led to their declining systemic and mesenteric flows. Observations from this current study of increasing afterload and decreasing mesenteric flows in the control animals is consistent with reports by others of progressive mesenteric A1 arteriolar vasoconstriction and decreasing mesenteric A1 arteriolar flow during resuscitation with saline and shed blood (Fruchterman et al., 1998).

Volume Of Lactated Ringer's Solution Required With Enalaprilat

The greater fluid requirement, as titrated by the mean arterial pressure in the enalaprilat group, would not be unexpected. One of the reasons to administer enalaprilat is to decrease mesenteric resistance arteriole constriction; thereby increasing perfusion in vascular beds that would otherwise remain constricted. Enalaprilat-induced relaxation of mesenteric resistance arterioles should not promote pooling of blood in mesenteric capacitance vessels. The pooling of blood in mesenteric capacitance vessels is a function of the contractile state of the mesenteric veins (Reilly et al., 2001), which are not as responsive to angiotensin II as are the mesenteric arteries (Yamazaki et al., 1991). The fluid and blood pressure results of this current study suggest that it would not be prudent to decrease mesenteric, and therefore total peripheral resistance, without provision of fluids to perfuse the opened vascular beds. They would also tend to suggest that bolus administration of enalaprilat would not be wise.

Volume Of Lactated Ringer's Solution In Controls

A different resuscitation protocol would be to administer a predetermined volume of lactated Ringer's solution rather than titrating the administration to achieve a predetermined blood pressure. Since a pressure titrated protocol was chosen, one could ask whether the same systemic blood flows could have been achieved in the control dogs as the enalaprilat dogs by simply forcing more lactated Ringer's solution rather than titrating its administration according to each animal's blood pressure? Possibly, but how much lactated Ringer's solution and at what rate should it have been empirically given? While some Advanced Trauma Life Support courses recommend administration of 3

times the shed blood volume as crystalloid (ATLS, 1997), the actual shed blood volume is rarely known in trauma patients and estimates of external blood loss by EMTs have been shown to be quite inaccurate (Patton et al., 2001). Additionally, Healey et al. (Healey et al., 2001) have suggested a much higher ratio, on the order of 10 to 1 for crystalloid to shed blood, should be used when dealing with very severe hemorrhage. In Iowa Methodist Medical Center's (Des Moines, IA) emergency department, fluid administration to trauma patients is governed by the clinician's assessment of the patient's condition and response to said fluids (also recommended in the ATLS course as more important than the "3 to 1 rule"), not by a magic ratio based on a guessed blood volume loss. Considering these factors and that two of the control dogs in our titrated resuscitation protocol achieved higher than target mean arterial blood pressures despite receiving the lowest volumes of lactated Ringer's solution, we are not comfortable with resuscitation protocols using one pre-set volume.

Consideration of afterload issues would also suggest that simply forcing more lactated Ringer's solution into each dog, independent of the effect on mean arterial blood pressure, might not be equally advantageous to administering enalaprilat while titrating fluid administration. The contractile state of resistance arterioles has a considerably greater influence on afterload and flow into a vascular bed than on venous return (Guyton et al., 1973). The state of the venous capacitance vessels has a considerably greater influence on venous return than on afterload. Prevention of angiotensin II formation with enalaprilat predominantly affects the state of the mesenteric resistance arterioles (Reilly et al., 2001; Yamazaki et al., 1991). Elevated angiotensin II levels have been observed in ICU patients for at least 6 days following admission (Boldt et al., 1995a). If this were

combined with administration of more intravenous fluid than necessary for maintenance of mean arterial pressure, one could increase the volume in the capacitance vessels without specifically impacting the contractile state of the mesenteric resistance arterioles. While preload would likely be increased, afterload would not be specifically reduced.

Comparison of Blood Flow Monitoring Techniques

Thermodilution determined cardiac output and combined M-mode and pulsed Doppler ultrasonically determined descending thoracic aortic blood flow both show a generally declining systemic blood flow in the control animals following the initial resuscitation-induced increase. They also both show higher ending systemic blood flows in the enalaprilat group. The thermodilution cardiac output profiles, however, do not match the celiac arterial flow profiles as well as the descending thoracic aortic flow profiles match the celiac arterial flow profiles. The nonpassive nature of the commonly used intermittent thermodilution technique may account in part for this difference (at least three 10 mL cold boluses per measurement in this study in dogs with average stroke volumes of 6 to 22 mL depending on the time point in the protocol). An additional reason for closer agreement between the descending thoracic aortic and the celiac arterial flows would be that these two sites should have a greater shared variance with each other than would entire cardiac output and celiac arterial flow. This is because cardiovascular insults lead to differential regulation of mesenteric blood flow versus cardiac and cerebral blood flow, which are not part of the descending thoracic aortic blood flow. This could be of benefit in monitoring trauma patients since direct monitoring of mesenteric perfusion is not generally done and since considerable evidence suggests that inadequate

mesenteric perfusion can lead to both gastrointestinal (Reilly et al., 2001) and systemic organ damage and dysfunction (Reilly et al., 2001; Kioke et al., 1994; Zallen et al., 1999; Magnotti et al., 1998).

As this study found, others have reported strong correlations between changes in descending thoracic aortic blood flows as measured ultrasonically with combined M-mode and pulsed Doppler technology and total cardiac output as measured by intermittent thermodilution, continuous thermodilution, and indirect calorimetry. The comparisons have been done in human patients as well as in experimental animals. Results from this study suggest that monitoring descending thoracic aortic blood flow rather than total cardiac output would be more useful for indicating of mesenteric flow.

Esophageal And Gastrointestinal P_iCO_2 During Resuscitation

Esophageal, gastric, and duodenal P_iCO_2 values increased during hemorrhage and decreased during resuscitation. Within the first 40 minutes of resuscitation, before the constant rate infusion of enalaprilat was started, the P_iCO_2 values at all the monitored sites in both groups decreased to or below the pre-hemorrhage values. Despite the declining celiac arterial blood flow in the control dogs, the P_iCO_2 values did not increase during resuscitation in the control dogs in the limited time course of this experiment. Whether differences in gastrointestinal P_iCO_2 would develop between the enalaprilat and control groups given a longer resuscitation time frame is currently under investigation.

Clinical Use Of Enalaprilat Post-Trauma

The systemic blood flow increases when enalaprilat is administered as a constant rate infusion during resuscitation observed in this study agree with the clinical findings reported by others who have used enalaprilat for reasons other than the treatment of hypertension. An increase in cardiac index was observed by Kincaid et al. (Kincaid et al., 1998) in 10 trauma patients who received 0.625 mg enalaprilat in 100 ml of saline as an infusion over 4 hours. Improvements in cardiovascular status were observed in critically ill trauma and postoperative patients when enalaprilat was given at a rate of 0.5 mg/hr over 5 days as compared to controls (Boldt et al., 1995b). In a subsequent study involving forty septic surgical patients, enalaprilat administration at 0.25 mg/hr over 5 days was not associated with an increase in cardiac index but was associated with a decrease in systemic vascular resistance index (Boldt et al., 1998).

Angiotensin Converting Enzyme Inhibition Versus Receptor Blockade

In addition to interrupting angiotensin II-induced splanchnic vasoconstriction, enalaprilat may be beneficial during resuscitation in other ways. In the study involving septic surgical patients (Boldt et al., 1998), enalaprilat administration was associated with lower circulating levels of thrombomodulin and soluble leukocyte and endothelial cell adhesion molecules, a decrease in neutrophil count, a decrease in lactate, and an improvement in ventilation to perfusion ratio (P_aO_2/F_iO_2). Fewer of the enalaprilat treated patients developed severe sepsis or septic shock (Boldt et al., 1998). In an animal skin fold ischemia-reperfusion model, enalaprilat prevented muscle cell death and

functional capillary density loss (Guba et al., 2000). In a tissue culture hypoxia-reoxygenation model, enalaprilat prevented up regulation of the endothelial cell adhesion molecules, ICAM-1 and VCAM-1, and neutrophil adherence to endothelial cells (Guba et al., 2000). Enalaprilat also blunted renal VCAM-1 up-regultation in a ureteral obstruction model (Morissey et al., 1998), and attenuated hepatic ischemia-reperfusion damage in a liver transplant model (Anthuber et al., 1997). Although the exact mechanisms resulting in these effects are still under study, it is clear that the effects noted in the ischemia-reperfusion and hypoxia-reoygenation experiments and in the ureteral obstruction experiments were not related to the prevention of angiotensin II interaction with the angiotensin II sub-type 1 receptor (Guba et al., 2000; Morrissey et al., 1998). Additionally, enalaprilat has been shown to decrease nuclear factor κB activation (Morrissey et al., 1997) and to decrease peripheral blood mononuclear cell production of TNFα and IL-1β by a posttranscriptional effect not present with all angiotensin converting enzyme inhibitors (Schindler et al., 1995). Additional work is needed to determine the role of enalaprilat as a modulator of the inflammatory consequences of hemorrhagic shock.

CONCLUSION

The data from this study demonstrates that enalaprilat has beneficial effects on systemic and mesenteric cardiovascular variables when administered during resuscitation in conjunction with intravenous lactated Ringer's solution. Further, these effects can be observed more accurately with continuous passive methods such as ultrasound than with intermittent active methods such as bolus-dependent thermodilution techniques.

Resuscitation in this model involved the administration of crystalloids only. The cardiovascular, metabolic, and immunomodulatory actions of enalaprilat should be investigated in a resuscitation model that includes the administration of banked packed red blood cells.

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ABBREVIATIONS

ABF	descending thoracic aortic blood flow measured by using combined M-mode and pulsed Doppler ultrasound
ad libitum	without restraint or limit
ASVI	aortic stroke volume index
ATLS	advanced trauma life support
BE	base excess
BE_a	arterial base excess
BE_v	mixed venous base excess
C	control group
CI	cardiac index
cm^3	centimeters cubed
CO	cardiac output
CO_2	carbon dioxide
CRI	constant rate of infusion of saline vehicle with or without enalaprilat
CVP	central venous pressure
dP_iCO_2	duodenal intraluminal P_iCO_2
DO_2I	oxygen delivery index
E	enalaprilat group
E_aI	arterial elastance index
ECG	electrocardiogram
EMT	emergency medical technician
enal	constant rate of infusion of enalaprilat

eP_iCO_2	esophageal intraluminal P_iCO_2
F_iO_2	fraction of inspired oxygen
Fr	French
gP_iCO_2	gastric intraluminal P_iCO_2
H	hemorrhage
Hb	hemoglobin
HR	heart rate
hr	hour
ICAM-1	intercellular adhesion molecule one
ICU	intensive care unit
IL-1β	interleukin 1 Beta
iP_iCO_2	ilium intraluminal P_iCO_2
kg	kilograms
L	liters
LRS	lactated Ringer's solution
LVPI	left ventricular power output index
MAP	mean arterial pressure
m^2	meters squared
mg	milligram
min	minutes
mL	milliliters
mmHg	millimeters of mercury
n	number of dogs in each group

NA	not available
nuclear factor κB	nuclear factor kappa B
p-value	objective statistical quantity for determining the viability of the null hypothesis
P_aCO_2	arterial PCO_2
P_aO_2	arterial PO_2
PAOP	pulmonary artery occlusion pressure
PAP	pulmonary artery pressure
pH_a	arterial pH
P_iCO_2	partial pressure of intraluminal carbon dioxide
R	Resuscitation
S_aO_2	fiber optically determined arterial saturation with O_2
SIMV	synchronous intermittent mandatory ventilation
SpO_2	pulse oximeter determined arterial saturation with O_2
Start CRI	start constant rate of infusion of saline vehicle with or without enalaprilat
Start R	start of resuscitation
SVI	stroke volume index
S_vO_2	mixed venous oxygen saturation
SWI	stroke work index
TNF_α	tumor necrosis factor alpha
VCAM-1	vascular cell adhesion molecule one
VO_2I	oxygen consumption index

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